Evidence that the [redacted]

The following data demonstrates that the [redacted] that is the subject of this MCAN [redacted] The data was generated from the ethanol facilities at [redacted] which is a customer of our prior business partner. The raw data and [redacted] with the [redacted].

[redacted]

[redacted]

As indicated in the above charts, 100% inactivation of the modified yeast is achieved at [redacted] when the modified yeast are treated at this temperature for a period of at least five minutes. Discussions with [redacted], another customer of our prior business partner, confirm that the residence time and temperature necessary to kill the modified yeast are representative of the conditions under which ethanol distillation and distillers' grains production occur. Specifically, [redacted], which utilizes an ethanol evaporator from [redacted], maintains a distillation temperature range [redacted] recirculation rate and a mean exposure frequency of eight times. Thus the total distillation exposure time is on average [redacted] Since each exposure cycle of [redacted] at temperatures [redacted] is greater than the 5 minutes necessary to kill 100% of the modified yeast, we conclude that all of the modified yeast will be inactivated under normal operating conditions.

In addition, yeast exposed to stresses, such as low pH from lactic and acetic acid due to bacterial contamination, temperature fluctuations, and high ethanol content, can cause the yeast to be even more sensitive to high temperatures under plant conditions compared to laboratory settings. These additional stresses have a synergistic effect, in which the sum total stress is collectively greater than the challenge presented by any of these conditions individually [redacted]

In second generation cellulosic ethanol plants the spent biomass from the stills is incinerated ensuring a greater than 6 log reduction in viable organisms.

Pre-distillation equipment conditions: The up-stream process equipment utilized for fermentation and transfer from the fermentor to the distillation column are cleaned after each batch via a Clean In Place (CIP) process.

Proper hygiene for the fermentor and other ethanol plant equipment is critical because bacteria and wild yeast contamination can significantly reduce ethanol production. Published figures for how often cleaning takes place are not readily available. However, we know that standard cleaning and hygiene protocols for ethanol facilities are lethal for most microbes and the modified yeast as shown by the lethality plots above. A description of typical hygiene practices in ethanol plants is provided in (Attachment 23) where it is noted that plants that operate above 160 °F are 'microbiologically safe'.

As represented in the MCAN, approximately 25-60% of the water used in the ethanol production process is recycled for the next fermentation; water that is not recycled will be sent to a waste water treatment facility. In the case of batch fermentation systems, in which the fermentor is emptied after each round of fermentation, such a process allows for a cleaning cycle after a batch (Attachment 23). While these vessels are monitored for microbial contamination, it could not be established that every ethanol producer will perform CIP after every batch. Nonetheless, when they are cleaned the conditions would be such that any yeast in the residue on the equipment would be inactivated by the combined action of the heat and detergents utilized in CIP.

Continuous fermentation in closed looped systems, in which the ferrnentor is not emptied, require a programmed shutdown for CIP implementation (Attachment 23). This can be an expensive undertaking and is largely avoided through the use of antimicrobial additives to control microbial contamination. CIP implementation in continuous fermentation systems usually occurs when microbial contamination becomes an issue and the CIP procedures should be sufficient to achieve 100% inactivation of the microbes, including yeast, remaining in the fermentor tank. In addition, the yeast in continuous fermentation systems have a fixed lifespan and must be repitched to maintain productive fermentation rates.

[redacted].